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BHARANGIN, A NOVEL DITERPENOID QUINONEMETHIDE FROM PYGMACOPREMNA HERBACEA (ROXB.) MOLDENKE[1]

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Abstract - Bharangin isolated from the hexane extract of the root nodules of the Ayurvedic drug. Pygmacopremna herbacea (Roxb.) Moldenke.(Verbenaceae) has been shown to be a novel diterpenoid quinonemethide incorporating a δ-lactone moiety (3) and possessing (S) configuration at the chiral carbon atom from a consideration of its mass spectrum,UV,IR, ^{1}H NMR and ^{13}C NMR spectra, 2D NMR INADEQUATE and CD spectrum. (3) appears to be derived biogenetically from a hypothetical diterpenoid skeleton designated as pygmane (4)

The root nodules of Pygmacopremna herbacea (Roxb.) Moldenke syn Premna herbacea Roxb. (Verbenaceae, Telugu Gantubharangi) is claimed to be useful in the Ayurvedic system of medicine for the treatment of several ailments[2] Column chromatography of the concentrated hexane extract of the powdered crude drug over silica gel yielded three compounds designated as bharangin[3], isobharangin and bharanginin and the structure elucidation of bharangin is reported

High resolution mass analysis of the molecular ion M^+, 328 1678 of bharangin, yellow crystalline needles, m p.213-14°(CCl_4),[α]$_D^{26}$ +350 3°($CHCl_3$,c,0 878) gave the elemental composition $C_{20}H_{24}O_4$(Calc.328 1674). Intense absorptions in the UV, visible spectrum (MeOH), λ_{max} 388 nm (Log ε 4.42) and IR spectrum (KBr)(ν_{max} 1598 cm^{-1} >C=O) of bharangin suggested the presence of an extended quinonemethide chromophore in its structure[4], which is confirmed by a carbon signal at 178.7 (> C=O,quinonemethide) in its ^{13}C NMR spectrum[5]. A hydroxyl group (ν_{max} 3320 cm^{-1}) attached to a quinonemethide chromophore and a δ-lactone moiety (ν_{max} 1740 cm^{-1}) present in the structure of bharangin are also indicated in its IR spectrum which are confirmed by the hydroxyl signal at 7.35 (exchangeable with D_2O) in its ^{1}H NMR spectrum and carbon signal at 169.5 in the ^{13}C NMR spectrum[6] respectively

All the twentyfour hydrogen atoms and twenty carbon atoms in its molecular formula are observed in its ^{1}H NMR spectrum and ^{13}C $\{^1H\}$NMR spectrum respectively ($CDCl_3$, chemical shifts in δ values with TMS as internal standard) The molecular formula, UV, IR and ^{1}H NMR spectra of bharangin compare closely with those of deoxyfuerstion (1) suggesting a diterpenoid skeleton incorporating an extended quinonemethide chromophore and a δ-lactone moiety in its structure. A notable difference in the ^{1}H NMR spectra of deoxyfuerstion (1) and bharangin is that the signals assignable to two sets of the methylene protons in the former are replaced by two AB quartets at 3 67,d(J=15Hz) and 2.88,d(J=15Hz) and 2 59,d(J=16Hz) and 2 45,d(J=16Hz) Furthermore, the chemical shifts and multiplicity of quaternary carbon signal at 83 9,s and the carbonyl signal at 169 5,s in the ^{13}C NMR spectrum of bharangin are reminiscent of C-8(83 5) and C-13(171 2) of ambreinolide (2)[6]. Thus, the methylene

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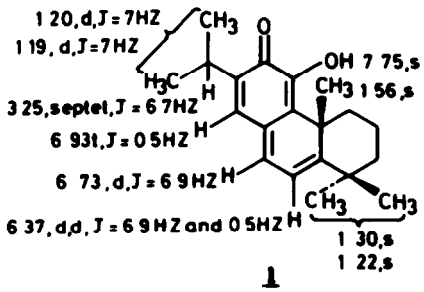

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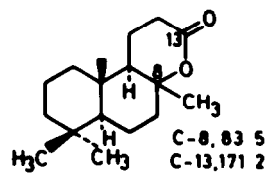

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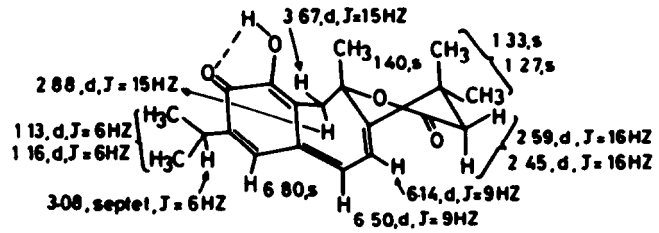

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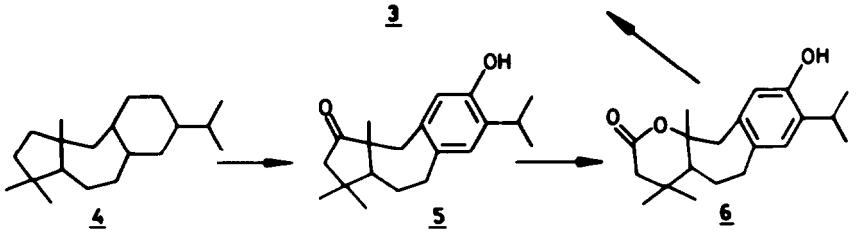

4 5 6

groups of ring A in deoxyfuerstion (1) appear to have been modified to a δ-lactone and a seven membered ring B. The carbonyl stretching absorption at 1740 cm^{-1} in the IR spectrum of bharangin suggests a preferred half chair conformation to the δ-lactone ring[7] and consequently, the seven membered ring also assumes a preferred conformation leading to structure (3) for bharangin. Finally, the structure of bharangin is unequivocally determined as (3) by the INCREDIBLE NATURAL ABUNDANCE DOUBLE QUANTUM TRANSFER EXPERIMENT (INADEQUATE)[8] leading to the CARBON-CARBON CONNECTIVITY 2D NMR PLOT (Fig.1) revealing the connectivities of all the adjacent carbon atoms as the natural abundance ^{13}C-^{13}C spin-spin coupled systems. It can be seen that the doublets are symmetric about a diagonal line The carbon peaks have been numbered from 1-20 beginning with the conjugated carbonyl at 178 71 to facilitate the interpretation of the carbon-carbon connectivities and therefore differ from the conventional numbers used in nomenclature Due to their long relaxation times, the signals for non-protonated carbons are weaker and do not always appear on the plot. The doublets for carbons-4 and 5 bonded to carbon-1 of the carbonyl appear on the figure underneath the carbon peaks-4 and 5 and they are found at the same distance to the right of the diagonal line. Although the doublets for carbon-1 are comparable to the noise level under the conditions of the experiment, the chemical bonds 1-4 and 1-5 can be inferred from the doublets arising from carbon-4 and carbon-5 Similarly, the bond 3-14 is defined by the position of doublet underneath the carbon-14 The region of the plot showing the connectivities 5-7,6-8 and 7-8 is rather compressed and they are clear in the expanded spectrum. Thus, the crucial quaternary carbon-11 at 83 9 adjacent to the oxygen atom of the lactone ring is coupled

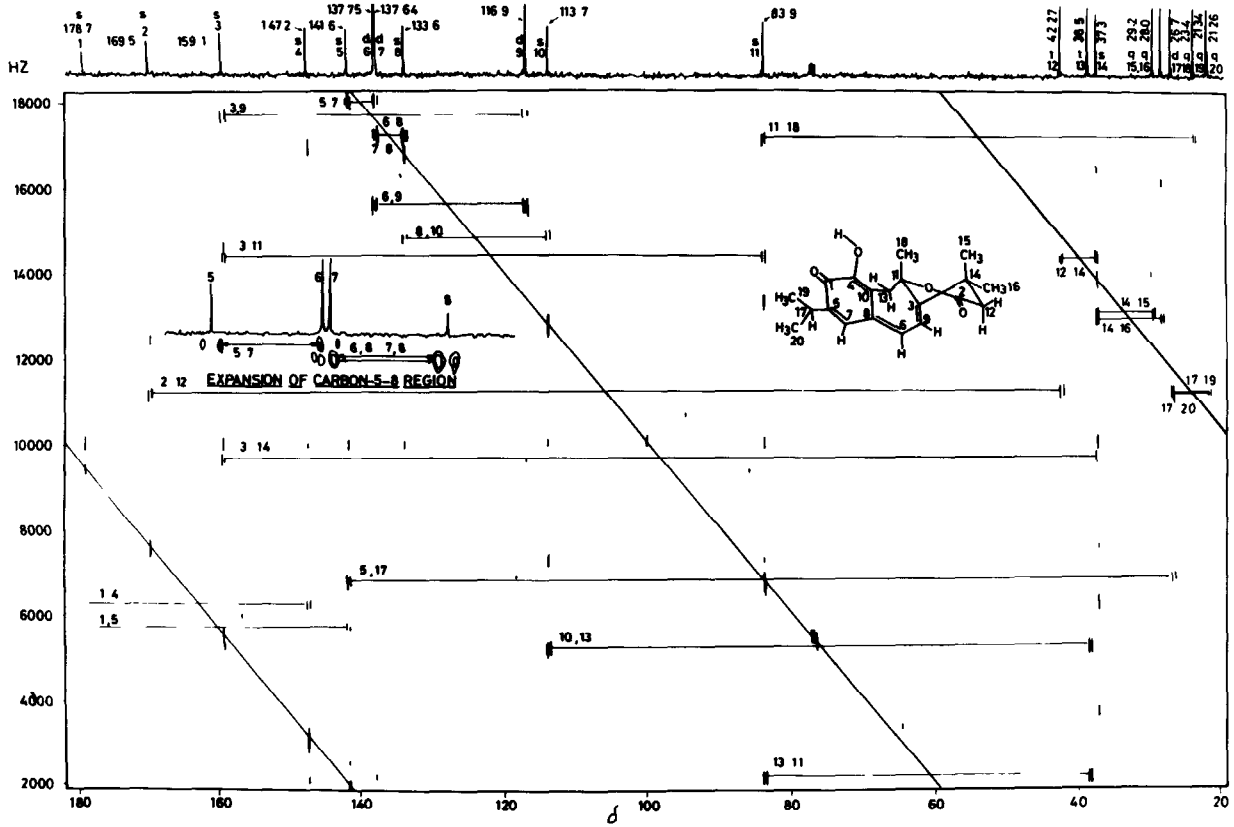

FIG 1 CARBON-CARBON CONNECTIVITY 2D-NMR PLOT (INADEQUATE)

The carbon-carbon connectivity experiment was carried out with XL 400 Varian NMR spectrometer at 100.557 MHz for ^{13}C using 270 mg of bharangin dissolved in 0 4 ml of $CDCl_3$ in a 5 mm tube at a temperature of 60°C (to keep it in solution) requiring 63 hours 40 8 minutes. The other parameters of the experiment are CCC2DQ Pulse Sequence[8]; Observe Carbon; Spectral Width 16393.5 Hz; Acq Time 0 125 Sec ;Delay time 4 5 Sec.;Decouple Proton;Data Processing;Pseudo-Echo Shaped;FT Size 4KX512 The multiplicities, determined by the APT (Attached Proton Test) experiment are shown for each peak as s (singlet), d (doublet), t (triplet) or q (quartet) The chemical bonds are indicated by drawing horizontal lines on the spectrum.

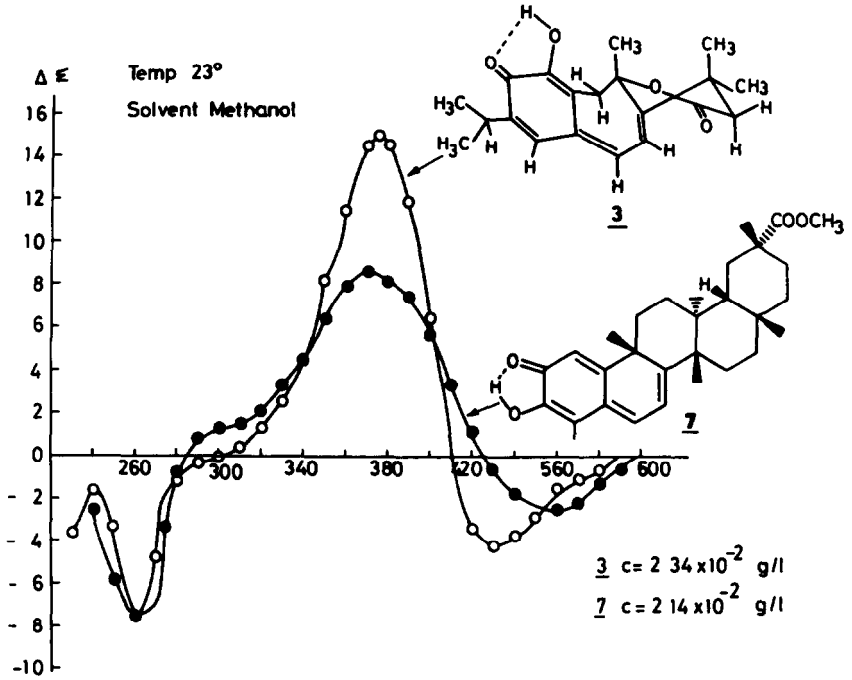

FIG 2 CD OF BHARANGIN AND PRISTIMERIN (JASCO MODEL J20 RECORDING SPECTROPOLARIMETER)

with the carbon atoms C-3 at 159 1, C-18 23 4 and C-13 38.5 The connectivities of each of these carbon atoms to the adjacent carbon atoms and so on, are obvious from the coupling patterns and they are self-explanatory The CD spectrum of bharangin (Fig.2) closely resembles that of pristimerin (7) establishing the absolute configuration (S) at the chiral carbon atom in its structure and furthermore, it shows approximately mirror image relationship to that of fuerstion[5].

Bharangin appears to be derived biogenetically from a novel diterpenoid skeleton, presently designated as pygmane (4), involving a number of oxidation steps One of the steps could be analogous to Baeyer-Villiger oxidation

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